or chloramphenicol palmitate working standard each into a 50-milliliter volumetric flask. Add approximately 35 milliliters of methanol and 1 milliliter of glacial acetic acid. Place in an ultrasonic bath for 10 minutes and dilute to volume with methanol.

(e) *Procedure.* Using the equipment, mobile phase, and operating conditions listed in paragraphs (a), (b), and (c) of this section, inject 10 microliters of the working standard solution into the chromatograph. Allow an elution time sufficient to obtain satisfactory separation of expected components. After separation of the working standard solution has been completed, inject 10 microliters of the sample solution into the chromatograph and repeat the procedure described for the working standard solution.

(f) *Calculations.* Calculate the chloramphenicol content as follows:

$$\frac{\text{Micrograms of chloramphenicol}}{\text{per milligram}} = \frac{(A)(W_s)(f)}{(B)(W_u)}$$

where:

A=Area of chloramphenicol palmitate sample peak (at a retention time equal to that observed for the standard);

B=Area of the working standard peak;

W_s=Weight of standard in milligrams;

W_u=Weight of sample in milligrams; and

f=Micrograms of chloramphenicol activity per milligram of chloramphenicol palmitate working standard.

[49 FR 6091, Feb. 17, 1984]

§ 436.336 Thin layer chromatographic identity test for azlocillin.

(a) *Equipment*—(1) *Chromatography tank.* A rectangular tank, approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, equipped with a glass solvent trough in the bottom and a tight-fitting cover for the top.

(2) *Iodine vapor chamber.* A rectangular tank approximately 23 centimeters long, 23 centimeters high, and 9 centimeters wide, with a suitable cover, containing iodine crystals.

(3) *Plates.* Use 20 x 20 centimeter thin layer chromatography plates coated with Silica Gel G or equivalent to a thickness of 250 microns.

(b) *Reagents*—(1) *Buffer.* Dissolve 9.078 grams of potassium phosphate, monobasic (KH_2PO_4) in sufficient distilled water to make 1,000 milliliters (solution A). Dissolve 17.88 grams of sodium phosphate, dibasic, heptahydrate ($Na_2HPO_4.7H_2O$) in sufficient distilled water to make 1,000 milliliters (solution B). Place 12.1 milliliters of solution B into a 100-milliliter volumetric flask and dilute to volume with solution A.

(2) *Developing solvent.* Place 50 milliliters of *n*-butyl acetate, 9 milliliters of *n*-butanol, 25 milliliters of glacial acetic acid, and 15 milliliters of buffer into a separatory funnel. Shake well and allow the layers to separate. Discard the lower phase and use the upper phase as the developing solvent.

(c) *Preparation of spotting solutions.* Prepare solutions of the sample and working standard, each containing 20 milligrams of azlocillin per milliliter in distilled water.

(d) *Procedure.* Pour developing solvent into the glass trough on the bottom of the chromatography tank to a depth of about 1 centimeter. Use the chamber immediately. Prepare plate as follows: Apply spotting solutions on a line 2.5 centimeters from the base of the silica gel plate and at points 2.0 centimeters apart. Apply approximately 10 microliters of the working standard solution to points 1 and 3. When these spots are dry, apply approximately 10 microliters of sample solution to points 2 and 3. Place spotted plate in a desiccator until solvent has evaporated from spots. Place the plate into the glass trough at the bottom of the chromatography tank. Cover the tank. Allow the solvent to travel about 15 centimeters from the starting line. Remove the plate from the tank and allow to air dry. Warm the iodine vapor chamber to vaporize the iodine crystals and place the dry plate in the iodine vapor chamber until the spots are visible, usually about 10 minutes.

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The azlocillin sample and the standard should have spots of corresponding R_f values (approximately 0.4), and standard and sample combined should appear as a single spot for

azlocillin. The penicilloate and penilloate of azlocillin as well as ampicillin appear as additional spots with R_f values of approximately 0.15, 0.3, and 0.25, reectively.

[47 FR 53348, Nov. 26, 1982]

§ 436.337 High-pressure liquid chromatographic assay for cephradine.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 8 millimeters;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder that is compatible with the detector output;

(5) A suitable integrator (optional); and

(6) A 25-centimeter column having an inside diameter of 4.6 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 10 micrometers in diameter, U.S.P. XX.

(b) *Reagents.* (1) 4 percent glacial acetic acid.

(2) 3.86 percent sodium acetate.

(c) *Mobile phase.* 4 percent glacial acetic acid:3.86 percent sodium acetate:methanol:distilled water (3:15:200:782). Filter the mobile phase through a suitable glass fiber filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase prior to its introduction into the chromatograph pumping system. The distilled water:methanol ratio may be varied to obtain acceptable operation of the system.

(d) *Operating conditions.* Perform the assay at ambient temperature with a typical flow rate of 1.2 milliliters per minute. Use a detector sensitivity setting that gives a peak height for the cephradine in the cephradine working standard that is about 75 percent of full scale.

(e) *Preparation of working standard and sample solutions*—(1) *Preparation of cephradine working standard solution.* Place an accurately weighed portion of the cephradine working standard into a suitably sized container. Add 5.0 milliliters of distilled water and place in an ultrasonic bath to facilitate dissolution. Dilute with a sufficient amount of mobile phase to obtain a solution containing 0.8 milligram of cephradine activity per milliliter.

(2) *Preparation of cephalexin working standard solution.* Dissolve an accurately weighed portion of the cephalexin working standard with mobile phase to obtain a solution containing 0.02 milligram of cephalexin activity per milliliter. Place in an untrasonic bath to facilitate dissolution.

(3) *Preparation of sample solutions*—(i) *Product not packaged for dispensing (micrograms of cephradine per milligram*). Dissolve an accurately weighed portion of the sample with mobile phase to obtain a solution containing 0.8 milligram per milliliter. Place in an ultrasonic bath to facilitate dissolution. Using this sample solution, proceed as directed in paragraph (f)(1) of this section.

(ii) *Product packaged for dispensing.* Determine both micrograms of cephradine per milligram of the sample and milligrams of cephradine per container. Use separate containers for preparation of each sample solution as described in paragraphs (e)(3)(ii) (*a*) and (*b*) of this section.

(*a*) *Micrograms of cephradine per milligram.* Dissolve an accurately weighed portion of the sample with mobile phase to obtain a solution containing 0.8 milligram per milliliter. Place in an ultrasonic bath to facilitate dissolution. Using this sample solution, proceed as directed in paragraph (f)(1) of this section.

(*b*) *Milligrams of cephradine per container.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with mobile phase to obtain a solution containing 0.8 milligram per milliliter. Using this sample solution,